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Experimental Aluminum Pathology in Rabbits: Effects of Hydrophilic and Lipophilic Compounds

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Aluminum lactate [Al(lact)$_3$] (hydrophilic, hydrolytically unstable) and aluminum acetylacetonate [Al(acac)$_3$] (lipophilic, hydrolytically stable) were tested as potential toxicants to rabbits upon IV administration both as aqueous solutions and as liposome suspensions. Both chemicals behaved as cardiotoxic agents when administered as aqueous solutions, but Al(acac)$_3$ was at least two orders of magnitude more active than Al(lact)$_3$. Al (acac)$_3$, but not Al(lact)$_3$, caused myocardial infarcts resembling those in humans (with contraction bands) at doses as low as 0.24 mg/kg body weight, as well as a prominent acanthocytosis. Al(lact)$_3$, when administered as a liposome suspension, was about 300 times more toxic than in aqueous solution, although cardiac damage was not infarctual in character. Both chemical and physical speciation of aluminum(III) thus play an essential role in determining the toxicity of the metal.

Introduction

Aluminum(III) is being actively investigated as a metal relevant to neurological disorders, such as Alzheimer's disease (*1,2*), dialysis dementia (*3*), osteomalacia (*4*), as well as being investigated for ecotoxicological effects (*5*). A possible role of aluminum uptake in the pathophysiology of dementias is suggested from recent epidemiological literature (*7*). However, from a biochemical point of view, the etiological connection between aluminum(III) and such pathologies remains an open question (*1*).

In spite of toxicological (*in vivo* and *in vitro*) (*6*), enzymological (*8*), and biochemical (*6,9*) work over the last decade, information on the reactivity of aluminum(III) with Lewis bases suitable for mimicking possible interaction(s) of the metal with receptor sites of biomolecules is scarce (*10,11*). Moreover, while considerable information is available on the metallo-organic chemistry of aluminum(III) (*12*), the aqueous chemistry of the ion in the presence of organic ligands at neutral pH is relatively unexplored. Virtually all biological work, thus, depends on the use of aqueous solutions in which the chemical identity (the speciation) of the employed toxin is rather ill-defined. Three main types of aqueous aluminum have been used so far: *a*) salts of strong acids (e.g., chloride and sulfate); *b*) salts of moderately strong acid with appreciable metal bonding ability (e.g., lactate and tartrate); and *c*) hydrolytically stable neutral complexes, i.e., aluminum acetylacetonate [Al(acac)$_3$] (acetylacetonate= 2,4-pentane-dionate) and aluminum maltolate [Al(malt)$_3$] (maltolate = 3-hydroxy,2-methyl,4-pyronate).

Solutions of type *a*, once adjusted to pH 7.5, produce quantitatively $Al(OH)_3$ (solubility equal to about 10^{-7} M). Among solutions of type *b*, those obtained from aluminum lactate appear to be stable at pH 7.5, but they are, in fact, probably metastable (*13*). In this case, the actual identity of biologically active species is a matter of speculation (*10*). Solutions of type *c* are currently in use in our laboratory and in other laboratories (*1,14*); preliminary results obtained with Al(acac)$_3$ (*14*) seem to indicate novel biological effects of this metal.

We report here on the first extensive toxicological work carried out with aluminum(III) solutions in which the nature of the administered toxin is either well known, i.e., Al(acac)$_3$, or at least can be reasonably proposed on the basis of preliminary new findings (*15*), i.e., aluminum lactate [Al(lact)$_3$] (lactate=2-hydroxy-propanoate). Data referring to the use of liposomes as carriers of both Al(acac)$_3$ and Al(lact)$_3$ are also reported.

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Materials and Methods

Animals and Chemicals

New Zealand white adult rabbits were obtained from Morini (Modena, Italy). Animals were maintained at controlled temperature and humidity and were supplied with water and standard rabbit food (Italiana Mangimi, Milano, Italy) *ad libitum*. The animals were anesthetized with Ketalar (Parke Davis) before being killed.

$Al(lact)_3$, (Fluka) and DL-dipalmitoylphosphatidylcholine (DPPC) (Sigma) were of reageant grade and used without further purification. $Al(acac)_3$ was prepared according to Yung and Reynolds (*16*) and was recrystallized from benzene/light petroleum. The purity of $Al(lact)_3$ and $Al(acac)_3$ was checked by elemental analysis. $Al(acac)_3$ was further controlled by ^{1}H-NMR and IR spectrometry. Acetylacetone (2,4-pentanedione) (Janssen) was distilled before use. Ultrapure water was prepared by distillation in a fused silica sub-boiling still and stored in polyethylene bottles. Reagent-grade concentrated nitric acid (Prolabo) was distilled in a sub-boiling still and stored in polytetrafluoroethylene (PTFE) bottles. Diluted nitric acid (1 M) was prepared by weight in a PTFE bottle.

Preparation and Analysis of Liposome Suspensions of $Al(acac)_3$

DPPC, 15 mg, and 105 mg of $Al(acac)_3$ (about 0.32 mmole) were dissolved in 2.1 mL of ethanol (95%) at 70°C with stirring. The resulting solution was slowly injected by means of a 50-μL microsyringe into a sterile NaCl solution (9 g/L, 30 mL) at 55 to 60°C. Each 50-μL portion was injected in 10 to 15 sec; during injections, the solution was stirred and the syringe needle was kept about 1 cm below the surface. The solution was allowed to cool under stirring and excess DPPC and $Al(acac)_3$ were removed by dialysis (cellulose membrane, ϕ = 1 cm, average pore diameter = 24 Å, exclusion limit 8000 to 15000 D, three treatments with 250 mL of physiological solution for 12 hr at 5° C). The final pH was about 7.0; the product had an opalescent appearance and was stored at 5° C in glass vials. Monolayer and single compartment characteristics of the liposomes were checked by a turbidimetric method (*17*) and confirmed by electron transmission micrography (*18*). The liposomes were found to be stable for about 1 month (turbidimetric test) (*17*).

The total aluminum content of the liposome suspensions was determined as follows: 0.5 mL of the suspension plus 1.0 mL of water were acidified with 0.2 mL of concentrated HNO_3 and kept at 80° C for 1 hr. The pH was adjusted at 7.4 by addition of 0.6 mL of aqueous NH_3 (25% w/w) and of 5 mL of borate buffer (0.18 M boric acid, 5 mM sodium tetraborate, pH 7.4). Aluminum was then determined spectrophotometrically as the 8-hydroxyquinolinate complex after extraction with toluene (*19*).

The aluminum content of the liposomes was determined as follows: 0.5 mL aliquots of the suspension were centrifuged (15 min at 30,000 rpm) to remove the aqueous phase, and the organic residual was treated with 1.5 mL of water and 0.2 mL of concentrated HNO_3; the resulting solution was analyzed as described above. The aluminum content of the liposomes was found to be about 50% of the total.

Preparation and Analysis of Liposome Suspensions of $Al(lact)_3$

The procedure and molar amounts were the same as for $Al(acac)_3$, with the exception that $Al(lact)_3$ was dissolved in the physiological solution to give a 0.05 M concentration; the alcoholic solution contained only DPPC.

As most of the aluminum was expected to be in the external aqueous phase, the dialysis procedure was modified as follows. During dialysis, the external solution was continuously renewed by a peristaltic pump operating at about 35 mL/hr; the external solution was sampled at various times, and its aluminum content determined by ionic chromatography. A bell-shaped concentration versus time profile was obtained, with the aluminum concentration dropping to negligible values (0.4–0.5 μM) after 5 days. The total aluminum content of the dialyzed suspensions was determined as described above; in view of the exhaustive nature of the dialysis procedure, the administered aluminum was assumed to be essentially all liposomes associated.

Administration Protocol, Chemoclinical Analyses, and Autoptical and Histological Evaluation

Animals were injected IV from the rostral auricolar vein under sterilite conditions. Solutions of $Al(lact)_3$ and $Al(acac)_3$ were sterilized by autoclaving at 110° C for 15 min. Control experiments were performed on three rabbits by injection of comparable amounts of free acetylacetone (aqueous solution, 300 μg/day for 16 days).

Urea, glucose, creatinine, total cholesterol, total bilirubin, alanine aminotransferase (ALAT/ GPT), aspartate aminotransferase (ASAT/GOT), alkaline phosphatase, gamma-glutamyltransferase (GGT), lactic dehydrogenase (LDH), creatine kinase (CK), total protein, and triglycerides were determined by means of Boehringer kits.

A thorough autoptical examination was performed on most animals. All macroscopic lesions were noted and samples of brain, spinal cord, heart, lungs, liver, spleen, kidneys, and skeletal muscle were examined microscopically after 24 to 48 hr fixation in 10% buffered formalin. Sections were stained with hematoxylin-eosin; brain and spinal cord were also examined with Bodian's stain; heart was examined with the Alcian-PAS and van Gieson methods.

Decontamination of Analytical Equipment

All the vessels employed for aluminum determination in tissues were decontaminated by washing with a detergent and then with diluted nitric acid and were kept immersed in diluted nitric acid. Fused silica test tubes were boiled in concentrated nitric acid for 1 hr. All the above items were carefully rinsed with ultrapure water before use and allowed to dry in a dust-protected box. Micropipette tips were decontaminated before use by loading twice with concentrated nitric acid and then with water.

Aluminum Determination in Tissues

Organs removed during the autoptic examination were stored at −20° C in decontaminated polystyrene vessels. A weighed tissue portion (2–5 g) was homogenized in a homemade PTFE potter (10 mL capacity) with 1 to 2 mL of water. The homogenate was then transferred to a decontaminated polypropylene test tube, freeze-dried, and stored at room temperature. The samples were dry ashed according to the following procedure (*20*): 150 to 300 mg of lyophilized material was directly weighed into a decontaminated 5-mL fused silica test tube and heated in a muffle furnace (temperature program: 1.5 hr each at 100, 150, 200, and 250° C; overnight at 480° C). After cooling, 1 mL of 1 M nitric acid was added, and the test tube was heated at approximately 100° C for 30 min. Occasionally, after this treatment, samples were found to contain small amounts of carbonaceous residue; in this case, the samples were treated in an ultrasonic bath for 1 hr and heated again as above. The samples were finally diluted with 1 mL of water and centrifuged. Aluminum was determined in the resulting clear solution by ion chromatography with colorimetric detection (Fig. 1), using the operating conditions shown in Table 1.

Results and Discussion

Chemical Nature of $Al(lact)_3$ and $Al(acac)_3$ in Water

Aluminum lactate has been frequently employed in toxicological research to prepare stock solutions of Al^{3+} ions. In

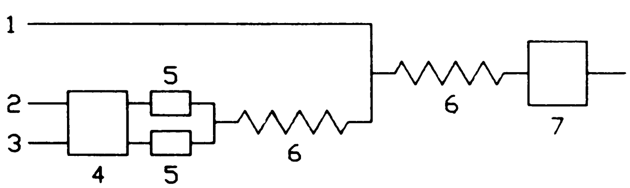

FIGURE 1. Detection system employed in the determination of aluminum by ion-chromatography. (1) Effluent from the analytical column; (2) buffer; (3) chromogenic agent; (4) peristaltic pump; (5) damper; (6) reactor coil; (7) colorimetric detector.

Table 1. Experimental details in the determination of aluminum by ion chromatography.

Eluent	0.15 M ammonium sulfate + 0.30 M ammonium nitrate + 0.10 M nitric acid; flow rate 0.70 mL/min
Chromogenic reagent	2 mM pyrocatechol violet + 8.8 mM nitric acid; flow rate 0.35 mL/min
Buffer	3.2 M hexamethylenetetramine + 44.0 mM nitric acid; flow rate 0.35 mL/min
Sampling loop volume	50 μL
Detector wavelength	570 nm

fact, this compound is reported to be "freely soluble in water" (*21*) and, unlike simple inorganic salts of Al^{III}, does not give rise to aluminum hydroxide precipitation upon neutralization and dilution. This observation is in contradiction with thermodynamic expectations based on a recent potentiometric investigation of the $Al^{III}/H_2O/lact^-$ system (*13*). In the pH range 3 to 5, the species $[Al(lact)H_2O)_4]^{2+}$, $[Al(lact)_2(H_2O)_2]^+$, $[Al(lact)_2(OH)(H_2O)]$, and $[Al(lact)_3]$ have been identified; the relevant stability constants clearly indicate that at physiological pH values, the total concentration of Al^{III} in equilibrium with solid $Al(OH)_3$ is unaffected by the presence of lactate.

The apparent contradiction between thermodynamic prediction and experimental fact can be explained either by the existence, at pH 7, of other, yet unknown, coordination compounds under equilibrium conditions, or the operation of kinetic factors. This latter kind of behavior is not unexpected for Al^{III} complexes under nonacidic conditions (*12*). Indeed, no aluminum-lactate complexes exist in appreciable amount at pH 7.5; rather, the predominant species are aquo-hydroxo aluminum species, metastable toward $Al(OH)_3$ formation (*15*). Based on available thermodynamic data (*22*), aqueous solutions of $Al(acac)_3$ should be stable at the concentration levels employed in this work. This was confirmed by the stability with time of the electronic spectra of the solutions.

Toxicity Data

A comparison of toxicity between aluminum lactate in aqueous solution ($Al(lact)_{3,\,aq}$) and the same chemical carried by liposomes ($Al(lact)_{3,\,lip}$) revealed that the latter is strongly (at least 300 times) more cardiotoxic than the former (Tables 2 and 3). For example, the data for animals 30 and 31 ($Al(lact)_{3,\,lip}$) and 18 and 19 ($Al(lact)_{3,\,aq}$) show that similar CK figures were reached with analytical amounts of administered toxin that differ by about two orders of magnitude. This prominent enhancement of toxicity for Al^{III} carried by the liposomes in their internal water micropools can be attributed to a biophysical speciation effect.

A liposome-based administration (likely to offer a more bioavailable form of Al^{III}) allows for discrimination between specific cardiotoxic effects of the metal and the rather nonspecific ones caused by the same species at far greater concentrations to brain, liver, kidneys, and lungs (Table 2). In particular, such effects appear to be prominent at $Al(lact)_3$ doses greater than 70 mg/kg body weight. Under these conditions, such massive effects could have led to secondary myocardial damage (e.g., edema).

The biological effect of $Al(acac)_{3,aq}$, estimated in terms of cardiotoxicity (Table 4), was also much higher (about 100 times) than that exhibited by $Al(lact)_{3,aq}$, but the type of cardiac damage was quite different. $Al(acac)_{3,aq}$ produced a myocardial effect, with myocardial necrosis (infarct), prominent contraction bands, and multifocal myocarditis (the protonated free ligand, acetylacetone, is nontoxic). A 4- to 5-fold increase of aluminum concentration in the cardiac tissue paralleled the histopathological and chemoclinical data. Details of this experimentally induced pathology have been

Table 2. Effects of $Al(lact)_3$ (aqueous solution).[a]

Rabbit no.[b]	Total amount of Al, mg[c]	Time of death[d]	Histopathological findings	Chemoclinical data	Al concentration in tissues, ppm[e]
9	139 (10 d, 7 i)	30 (killed)	Brain: no lesions Heart: dilated ventricles; focal round-cell inflammation Liver: focal round-cell and leukocytes (eosinophils) inflammatory infiltrate in lobular and portal tracts Kidneys: interstitial nephritis Lungs: acute stasis	ND[f]	Cortex: 2.2 Cerebellum: 4.2 Thalamo-mesencephalon: 1:1 Liver: 82 Kidneys: 20 Heart: 9.2
13	240 (15 d, 12 i)	0	Heart: massive dilatation, particularly of the right ventricle; interstitial edema and myocardial fiber disarray Liver: massive centrilobular necrosis with megakaryocytes and erythroblasts Kidneys: severe glomerulopathy with fibrin and basophilic granular material in capillaries; tubular necrosis; extramedullary hematopoiesis	ND	ND
14	350 (10 d, 5 i)	0	Brain: no lesions Heart and liver: as for rabbit no. 13	ND	ND
16	328 (7 d, 4 i)	0		ND	ND
18	130 (30 d, 21 i)	2 (killed)	Brain and heart: as for rabbit no. 9	CK[g]: increase up to 530–890 U.I.; other indexes normal	ND
19	88 (15 d, 11 i)	2 (killed)		CK: increase up to 570–800 U.I.; other indexes normal	ND

[a]pH adjusted to 7.5 with NaOH; injected volumes: from 0.5 to 2 mL of ~ 0.5 M solution.
[b]Numbers refer to pound registry.
[c]Number of days of treatment (d) and number of injections (i) in parentheses.
[d]Number of days after the last injection.
[e]Average Al concentration, ppm, in controls: total brain, 0.31 ± 0.1; cortex, 0.16 ± 0.03; cerebellum, 0.32 ± 0.12; thalamo-mesencephalon, 0.29 ± 0.14; heart, 0.61 ± 0.26; liver, 0.26 ± 0.03; kidney, 0.54 ± 0.25.
[f]ND, not determined.
[g]CK, creatine kinase. Normal CK value: 330 ± 76 U.I. (*23*).

Table 3. Effects of $Al(lact)_3$ (liposome suspension).[a]

Rabbit no.[b]	Total amount of Al, mg[c]	Time of death[d]	Histopathological findings	Chemoclinical data
30	0.27 (50 d, 42 i)	2 (killed)	Heart: interstitial hyperplasia and fibroblastic proliferation Brain, liver, kidneys, spleen, lungs and skeletal muscles: no lesions	CK[e]: gradual increase up to — 1000 U.I. at the end of treatment
31	0.02 (7 d, 4 i)	2 (killed)	Heart, brain, liver, kidneys, spleen, lungs, and skeletal muscles: as for rabbit no. 30 Spinal cord: large infarction of the lumbar tract, probably from ischemic damage	As for rabbit no. 30
34	0.07 (15 d, 11 i)	2 (killed)	Heart, liver, kidneys, lungs, and skeletal muscles: as for rabbit no. 30 Spinal cord: as for rabbit no. 31	CK and LDH: slight irregular increase

[a]pH 7 (spontaneously reached after dialysis); injected volumes: 4 mL of a ~ 0.06 mM suspension.
[b]Numbers refer to pound registry.
[c]Total amount, including aqueous and liposome carried Al^{III} (see text). Number of days of treatment (d) and number of injections (i) in parentheses.
[d]Number of days after the last injection.
[e]CK, pyruvate kinase; LDH, lactate dehydrogenase.

reported (*14*). It is worth noting that skeletal muscles undergo only indirect (secondary) damages.

The quite different biological effects on the same organ observed in the two cases clearly points to the importance of the speciation (i.e., the nature of the coordination sphere) of the administered metal. Cardiac muscle appears to be a target organ for aluminum (under pathologic conditions) for uremic patients (*3*). It is apparent that the chemical properties acquired by aluminum(III) in its peculiar speciation as $Al(acac)_3$ (hydrolytic stability, resistance to scavenging,

Table 4. Effects of $Al(acac)_3$ (aqueous solution).[a]

Rabbit no.[b]	Total amount of Al, mg[c]	Time of death[d]	Histopathological findings	Chemoclinical data	Al concentration in tissues, ppm[e]
26	0.8 (13 d, 8 i)	0	Brain: no lesions Heart: dilatation, particularly of the right ventricle; multifocal degeneration of myocardial fibers, with myocytolysis and contraction bands; interstitial edema and inflammation Liver: massive stasis, with centrilobular necrosis Kidneys: stasis; some eosinophils in glomerular capillaries Lungs: acute stasis and edema Skeletal muscles: no lesions	CK[f]: regular increase up to 1650 U.I. (after eight injections) LDH: slight and irregular increase	ND[g]
27	0.7 (13 d, 7 i)	0	As for rabbit no. 26	As for rabbit no. 26	ND
35	0.7 (20 d, 15 i)	0	As for rabbit no. 26	CK: irregular increase up to ~ 1000 U.I. after each injection LDH: slight and irregular decrease	Brain: 0.31 Heart: 1.7 Liver: 1.6 Kidney: 1.6
36	0.7 (20 d, 15 i)	2	As for rabbit no. 26	As for rabbit no. 35	Brain: 0.6 Heart: 2.1 Liver: 2.2 Kidney: 1.0
37	0.7 (20 d, 15 i)	0	As for rabbit no. 26	As for rabbit no. 35	Cortex: 0.08 Cerebellum: 0.79 Thalamo-mesencephalon: 0.18 Heart: 1.3 Liver: 1.9 Kidney: 1.0
38	0.7 (20 d, 15 i)	1 (killed)	Brain, heart, liver, kidneys as for rabbit no. 26	As for rabbit no. 35	Cortex: 0.28 Cerebellum: 0.26 Thalamo-mesencephalon: 0.33 Heart: 1.45 Liver: 2.4 Kidney: 1.1
39	0.7 (20 d, 15 i)	50 (killed)	Brain: no lesions Heart: focal fibrous scars, with some calcified cells Blood: development of acanthocytes	CK: as for rabbit no. 35; gradual return to the normal figure upon stopping the treatment[h] LDH: as for rabbit no. 35; gradual return to the normal figure upon stopping the treatment	ND
40	0.7 (20 d, 15 i)	20	Brain, heart, and blood as for rabbit no. 39	As for rabbit no. 39	Cortex: −0.08 Cerebellum: 0.44 Thalamo-mesencephalon: 0.16 Heart: 1.8 Liver: 2.6 Kidney: 0.53

[a]pH 7; injected volumes from 0.5 to 1.5 mL of a ~ 2.5 mN $Al(acac)_3$ solution.
[b]Numbers refer to pound registry.
[c]Number of days of treatment (d) and number of injections (i) in parentheses.
[d]Number of days after the last injection.
[e]Average Al concentration, ppm, in controls: total brain, 0.31 ± 0.1; cortex, 0.16 ± 0.03; cerebellum, 0.32 ± 0.12; thalamo-mesencephalon, 0.29 ± 0.14; heart, 0.61 ± 0.26; liver, 0.26 ± 0.03; kidney, 0.54 0.25.
[f]CK, creatine kinase; LDH, lactate dehydrogenase.
[g]ND, not determined.
[h]Normal CK value: 330 ± 76 U.I. (*23*).

and lipophilicity) (*24*) facilitate the action of this artificial toxin not only in reaching the organ, but also in interfering with receptor(s) that are essential to myocardial cell function (*14*).

The similarity of the cardiotoxic effect of $Al(acac)_{3,aq}$ and $Al(acac)_{3,lip}$ (Table 5) might seem surprising, in that a possible synergic action of the lipophilic vehicle with the lipophilic character of the coordination sphere could have been expected. Evidently, the above-mentioned properties of $Al(acac)_3$ make this complex sufficiently well suited in itself for selectively producing an infarctual cardiac lesion, such that the liposomal vehicle does not provide a significant synergic contribuution. The appreciable accumulation of

Table 5. Effects of $Al(acac)_3$ (liposome suspension).[a]

Rabbit no.[b]	Total amount of Al, mg[c]	Time of death[d]	Histopathological findings	Chemoclinical data
5–8	0.8 (10–15 d, 7–10 i)	0	Heart: dilatation, particularly of the right ventricle; multifocal myocytolysis; interstitial edema and inflammation Liver: stasis and mild steatosis Kidneys: stasis; some eosinophils in glomerular capillaries Spleen: congestion	ND[e]
22	0.6 (20 d, 15 i)	2 (killed)	As for rabbits nos. 5–8	CK[f]: 2-fold increase LDH: 3-fold increase at the end of treatment
23	0.6 (20 d, 15 i)	2 (killed)	As for rabbits nos. 5–8	CK: 10-fold increase LDH: 5-fold increase at the end of treatment

[a] pH 7; injected volumes from 0.5 to 1.5 mL of a ~ 3 mM $Al(acac)_3$.
[b] Numbers refer to pound registry.
[c] Total amount injected, including aqueous and liposome carried Al^{III} (see text). Number of days of treatment (d) and number of injections (i) in parentheses.
[d] Number of days after the last injection.
[e] ND, not determined.
[f] CK, creatine kinase; LDH, lactate dehydrogenase.

aluminum in liver and kidney (Tables 2 and 4) is not surprising, in view of the specific detoxicating functions carried out by these organs.

Inspection of Table 4 reveals that rabbit 39 survived after treatment for 20 days with $Al(acac)_{3,aq}$. In order to confirm the irreversible character of the artificial myocardial infarct induced by $Al(acac)_3$ (*14*), the animal was monitored for an additional 50 days. Although CK and LDH indexes returned to normal values after 3 days, histopathological analysis revealed that infarcted myocardial areas had undergone substitution of the affected fibers by collagen-rich scar tissue (Fig. 2). A similar pattern was observed also for rabbit 40, which died spontaneously 20 days after the end of treatment.

Interestingly, microscopic (SEM) observations on blood samples from animals 39 and 40 seven days after termination of treatment revealed a marked morphological anomaly of the erythrocytes (acanthocytosis) (Fig. 3). This anomaly, which was reported as associated with a form of encephalopathy in humans (*25*), may be interpreted as the consequence either of a damage to the emathopoietic system or of a direct action of Al^{III} on the erythrocyte membrane (*9,26*). An *in vitro* investigation appears to support this latter hypothesis (*27*).

As to the effect of $Al(lact)_3$ on the central nervous system, this toxin was rather ineffective in producing appreciable damage when administered as an aqueous solution, in contrast with the observed conspicuous metal accumulation in the brain (Table 2). On the contrary, treatment with liposome-carried $Al(lact)_3$ gave, in two cases (Table 3), posterior paraplegia with a wide infarcted area in the spinal cord.

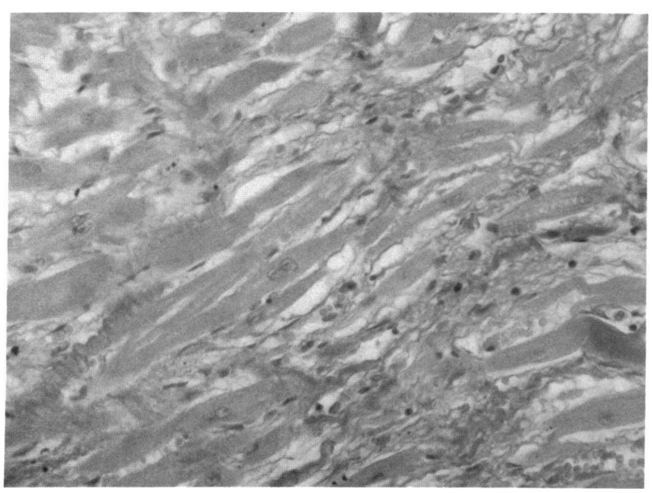

FIGURE 2. A collagen-rich tissue scar replacing a formerly infarcted myocardial area. van Gieson staining; × 750. (The dark areas correspond to the red ones in van Gieson staining.)

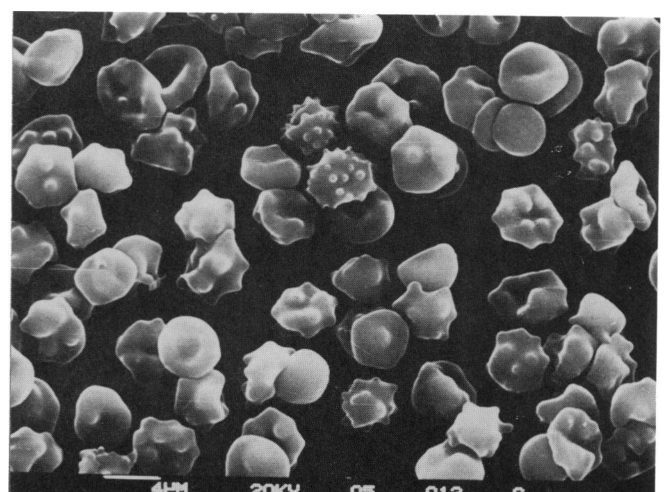

FIGURE 3. Scanning electron microphotograph of erythrocytes from rabbits treated with $Al(acac)_3$.

No neurological effects were observed with $Al(acac)_3$.

In general terms, the data reported here do not seem to indicate any connection between the IV injection of hydrolytically stable (lipophilic) $Al(acac)_3$ or of hydrolytically (meta)stable (hydrophilic) $Al(lact)_3$ and neurological disorders. The appparent contrast between our results and the neurological disorders with neurofilament degeneration observed upon SC administration of $Al(lact)_3$ (*28–30*) may simply be the result of different administration protocols. In the case of $Al(acac)_3$, the lack of effect may be due to the relatively short treatment times employed so far, together with an overwhelming cardiotoxic activity of the drug. It is possible that much lower doses and prolonged treatment times will lead to the production of neurologic effects.

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